# GAMMA HERPESVIRUS DNA AND METHODS OF USE

a A1 This application claims the benefit of U.S. Provisional Application 60/168,532, filed 2 December 1999, and U.S. Provisional Application 60/142,736, filed 8 July 1999, the disclosures of which are hereby incorporated by reference in their entirety.

## FIELD OF THE INVENTION

The present invention relates to newly identified polynucleotides, polypeptides, and fragments thereof encoded by porcine gamma-herpesvirus sequences, and methods of using the porcine gamma-herpesvirus nucleic acids and polypeptides.

## BACKGROUND OF THE INVENTION

Organ procurement currently poses one of the major problems in solid organ transplantation, since the number of patients requiring transplants far exceeds the number of organs available. One means of eliminating the shortage of donor organs for allotransplantation is to develop the technologies required to transplant non-human organs into humans, i.e., xenotransplantation. The development of clinical xenotransplantation will also allow for the transplantation of non-human cells and tissues.

A potential problem lies in the fact that human and animal organs may be of very different size, depending on the species serving as donor, and on the possibility of infection due to microorganisms present in the donor tissues and having an ability to infect humans. Consequently, one strain of the domesticated pig, denoted miniature swine (*Sus scrofa*), appears suitable for such transplants because of its similar size to humans (see below). Furthermore, any use of pigs as organ donors in xenotransplantation would obviate problems associated with the consideration of non-human primates as donors. Xenografts from non-human primates, for example, present considerable risk of transmission of pathogens and the consequent development of emerging infections. In addition, several pathogens that cause disease are known to infect both humans and non-human primates, for example, in the transmission of HIV from the chimpanzee to humans. Furthermore, chimpanzees and orangutans, the closest non-human primates phylogenetically, are endangered species and far too rare to be considered for organ transplantation purposes. Baboons are too small to be an appropriate donor for most organ transplants. Even the largest baboons weigh less than 40 kg. In addition, the gestation times and productivity of primates would not allow a commercially significant generation of source animals.

The physiology of many organ systems of pigs has been shown to be highly similar to the human counterparts (Sachs, D.H. (1994) *Veterinary Immunology & Immunopathology* **43**:185-191). Thus, the miniature swine offers numerous advantages as potential xenograft donors. They achieve adult weights of approximately 100 - 150 kg, a size that is more compatible to human weights than that of the domestic pig, which reaches weights of over 500 kg. Through a selective breeding program over the past 20 years, partially inbred, miniature swine have been produced (Sachs et al. (1976) *Transplantation* **22**: 559-567; Sachs, D.H. (1992) In *Swine as models in biomedical research*, eds M. Swindle, D. Moody, and L. Phillips, pp. 3-15. Ames Iowa State Univ. Press; Sachs, (1994) *Veterinary Immunology & Immunopathology* **43**: 185-191). This breeding program has resulted in herds of animals that are genetically well characterized

and inbred at the major histocompatibility complex (MHC). These animals have been used in large animal model studies for many years and have, like their domestic counterparts, very favorable breeding characteristics for being used as donors of organs in xenotransplantation.

A central concern regarding xenotransplantation is the risk of xenosis, infection by organisms transferred with the xenograft into both the transplant recipient and the general population. In particular, “emerging infections” caused by new and previously unknown infectious agents with altered pathogenicity, have to be considered as a potential risk associated with xenotransplantation. The risk of viral infection is increased in transplantation by the presence of factors commonly associated with viral activation, e.g., immune suppression, graft-versus-host disease, graft rejection, viral co-infection, and cytotoxic therapies.

Herpesviruses are the causative agents of many diseases that share a commonality of latency and recurrent infections. Herpesviruses may persist for years in a dormant state and become reactivated after later provocation. While the herpesviruses are widely separated in terms of genomic sequence and proteins, many are similar in terms of virion structure and genome organization. Herpesvirus represents a DNA virus family containing a central icosahedral core of double-stranded DNA. There is a lipoprotein envelope that is trilaminar and 100-200 nm in diameter and a nucleus that is 30-43 nm in diameter. The genome size is large, up to 235 kbp DNA. Based upon the structural and morphological features, the herpesvirus family is divided into three main families: alpha, beta, and gamma. Examples of alpha herpesviruses are herpes simplex and varicella zoster, examples of beta herpesviruses are cytomegalovirus and human herpesvirus 6 while examples of gamma-herpesviruses are Epstein Barr virus and human herpesvirus 8.

Prior to this invention, members of three porcine herpesvirus families had been identified, namely of the alpha, beta, and gamma-herpesvirus families. Suid herpesvirus 1 (SHV1), which causes pseudorabies (PRV) in pigs, is an alpha-herpesvirus and results in neonatal death of piglets, and can be eradicated by vaccination. The glycoprotein II gene of SHV1 is reportedly closely related to the gpB gene of other herpesviruses (Robbins et al. (1987) *J. Virology*. **61**:2691-2701). Suid herpesvirus 2 (SHV2), also known as pig cytomegalovirus (pCMV), is found in the respiratory tract of pigs and causes atopic rhinitis, abortion, or neonatal piglet losses. Only the DNA polymerase gene of SHV2 has been reported (Genbank Accession Number AJ222640). Detection of two novel porcine herpesviruses with high similarity to other gamma-herpesviruses were recently reported (Ehlers et al. (1999) *J. General Virology*, **80**:971-978), wherein the sequence of the DNA polymerase gene was reported (Genbank Accession Numbers AF118399 and AF118401).

Subsequent examination, as disclosed herein, of pigs for the presence of a gamma-herpesvirus by PCR methods designed to amplify the DNA regions encoding all or part of the glycoprotein B (gpB) envelope molecule has resulted in the detection of sequence similarity to other known gamma-herpesviruses.

## BRIEF SUMMARY OF THE INVENTION

It is an object of the present invention to provide isolated polynucleotide sequences encoding a polypeptide that corresponds to a novel porcine gamma-herpesvirus glycoprotein B, herein called pGHV-gpB. Such sequences may be derived from genomic DNA.

It is another object of the present invention to provide immunogenically active fragments and segments of said polynucleotide for use as probes in the detection of similar sequences in related organisms.

A further object of this invention is to use the polypeptides and fragments thereof of the invention to provide a vaccine against porcine gamma-herpesvirus organisms, which vaccine is useful to protect a pig from productive proliferation of this, or related, gamma-herpesvirus organisms.

A still further object of the present invention is to provide antibodies that are capable of binding to an epitope on the porcine gamma-herpesvirus gpB polypeptides, and fragments, of the invention. Such antibodies are useful for diagnosis of the presence of pGHV-gpB polypeptides or as part of a vaccination program.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the alignment of Glycoprotein-B (gpB) protein sequences from several known gamma-herpesviruses. The following gamma-herpesviruses were used for the analysis: human herpesvirus 8 (HHV8PEP; Genbank accession number AF092928), rhesus monkey rhadinovirus (RHESRHADPEP; Genbank accession number AF029302), murine herpesvirus 68 (MURH68PEP; Genbank accession number U97553), bovine herpesvirus 4 (BOVINEH4PEP; Genbank accession number Z15044), ateline herpesvirus 3 (ATELINEH3PEP; Genbank accession number AF083424), herpesvirus saimiri (SAIMIRIPEP; Genbank accession number X64346), equine herpesvirus 2 (EQH2PEP; Genbank accession number U20824), Epstein-Barr virus (EBVPEP; Genbank accession number V01555), Alcelaphine herpesvirus 1 (ALCELPEP; Genbank

accession number AF005370), and equine herpesvirus 5 (EQH5PEP; Genbank accession number AF050671). Degenerate primers were designed for conserved regions (underlined) along with specific primers for Epstein-Barr Virus (EBV) for control and optimization purposes. Such sequences are continued through Figures 1(a), 1(b) and 1(c).

Figure 2 shows the DNA sequence of the pGHV-gpB gene (SEQ ID NO: 23) that encodes a gamma-herpesvirus gpB polypeptide of the present invention. A fragment of this is shown as SEQ ID NO: 1.

Figure 3 shows the deduced polypeptide sequence of the pGHV-gpB cDNA shown in Figure 2 (SEQ ID NO: 24). The amino acids of the sequence are represented by standard one-letter codes. A fragment of this is shown as SEQ ID NO: 2.

Figure 4 shows a comparison of the nucleic acid sequences of pGHV-gpB and SHV1 and is therefore an illustration of the nucleic acid sequence identity between SEQ ID NO: 1 (a portion of the sequence of Figure 2) and a portion of Suid herpesvirus 1 (SHV1, Genbank accession number M17321 nucleotides 641-1300). Row 1 (pGHV-gpB DNA) of the compared sequences is SEQ ID NO: 1 (a portion of the sequence of Figure 2), row 2 is SHV1 (pGHV1 in the figure), nucleotides 641-1300, and row 3 indicates the nucleotides that show identity. Dashes indicate gaps that were inserted in the alignment process to maximize sequence identity.

Figure 5 is a comparison of the protein sequences of pGHV-gpB and SHV1 and thus an illustration of the identity between the deduced amino acid sequence of SEQ ID NO: 2 (a portion of the sequence of Figure 3) and SHV1. The amino acids of the sequence are represented by standard one-letter codes.

Row 1 of the compared sequences is SEQ ID NO: 2, row 2 is the amino acid sequence of SHV1 (pGHV1; amino acids 491-850) and row 3 indicates the amino acids that show identity. Dashes indicate gaps that were inserted in the alignment process to maximize sequence identity.

Figure 6 is a comparison of the nucleic acid sequences of pGHV-gpB and SHV2 and illustrates the nucleic acid sequence identity between SEQ ID NO: 1 and a portion of suid herpesvirus 1 (SHV2, Genbank accession number AJ222640). Row 1 of the compared sequences is SEQ ID NO: 1, row 2 is SHV2, and row 3 indicates the nucleotides that show identity. Dashes indicate gaps that were inserted in the alignment process to maximize sequence identity.



Figure 7 shows a comparison of the protein sequences of pGHV-gpB and SHV2 and illustrates the identity between the deduced amino acid sequence of SEQ ID NO: 2 and that of SHV2. The amino acids are represented by standard one-letter codes. Row 1 of the compared sequences is SEQ ID NO: 2, row 2 is the amino acid sequence of SHV2, and row 3 indicates the amino acids that show identity. Dashes indicate gaps that were inserted in the alignment process to maximize sequence identity.

Figure 8 is an illustration of the nucleic acid sequence identity between SEQ ID NO:1 and a portion of the porcine gamma-herpesvirus polymerase (AF118399). Row 1 of the compared sequences is SEQ ID NO:1, row 2 is AF118399 and row 3 indicates the nucleotides that show identity. Dashes indicate gaps that were inserted into the alignment process to maximize sequence identity.

Figure 9 is an illustration of the nucleic acid sequence identity between SEQ ID NO:1 and a portion of the porcine gamma-herpesvirus polymerase

(AF118401). Row 1 of the compared sequences is SEQ ID NO:1, row 2 is AF118401 and row 3 indicates the nucleotides that show identity. Dashes indicate gaps that were inserted into the alignment process to maximize sequence identity.

Figure 10 shows a Blast 2 sequence comparison of the nucleic acid sequence of pGHV-gpB and Acelaphine herpesvirus (GenBank Accession No. AF005370). The vertical lines indicate matches between the two sequences. The upper "Query" sequence represents the gpB nucleotide sequence while the lower "subject" sequence is the Acelaphine herpesvirus sequence. The numbers for the upper sequence correspond to the residue numbers shown for the sequence of Figure 2 (SEQ ID NO: 23). About 76% of the residues matched.

Figures 11 (a) and (b) show a comparison of the protein sequences of pGHV-gpB and Acelaphine herpesvirus (GenBank Accession No. gi/2337975 (AF005370). The amino acids of these sequences are represented by the standard one-letter codes. Row 1 (query) of the compared sequences is the pGHV-gpB while the lower row (the "subject" sequence) is the Acelaphine herpesvirus sequence. The numbers for the upper row correspond to the residue numbers shown in Figure 3 (SEQ ID NO: 24).

## DETAILED DESCRIPTION OF THE INVENTION

In accordance with one aspect of the present invention, there is disclosed herein an isolated polynucleotide which encodes the polypeptide comprising the amino acid sequence of SEQ ID NO: 24, corresponding to the gpB envelope protein of porcine gamma herpesvirus. Also disclosed are fragments of these

polynucleotide and polypeptide sequences, especially that of SEQ ID NO:1 (polynucleotide) and SEQ ID NO:2 (polypeptide).

Polynucleotide sequences of the present invention have been isolated from genomic DNA of miniature swine. These sequences show only low sequence similarity with other known porcine herpesvirus sequences (SHV1 and SHV2 and gamma-herpesvirus polymerase gene), including the sequences corresponding to Genbank Accession Numbers M17321, AJ222640, AF118399 and AF118401, respectively.

In accordance with a further aspect of the present invention the nucleic acid sequences of SEQ ID NO: 23, including fragments thereof, may be utilized under stringent hybridization conditions to isolate from porcine tissue by procedures known in the art, DNA sequences corresponding to porcine gamma-herpesvirus gpB regions and for complete porcine gamma-herpesvirus sequences.

Fragments of the polynucleotide sequences of the present invention were used as hybridization probes for a cDNA or DNA library to isolate the full-length gamma-herpesvirus sequence or fragments thereof. Such fragments also find use as probes in identifying other similar sequences of related organisms. Thus, the present invention further provides an isolated porcine gamma-herpesvirus polynucleotide fragment that is capable of stringently hybridizing to a porcine gamma-herpesvirus polynucleotide sequence. In this manner, the present invention provides probes and/or primers for use in *ex vivo* porcine gamma-herpesvirus detection studies. Typical detection methods involve use of the polymerase chain reaction, sequence analysis, and hybridization techniques. Thus, the present invention also provides pGHV-gpB specific oligonucleotide probes and primers.

The present invention further relates to a method of detecting the presence of gamma herpesvirus in a sample comprising detecting the presence in said sample of a polynucleotide having a sequence at least 80%, preferably at least 90%, most preferably 95% identical to a sequence encoding a polypeptide of the present invention. Said sample may be blood or other tissue sample. The presence of a polypeptide, or immunogenic fragments thereof, of the present invention may also be detected in such samples.

In addition, the present invention also relates to an isolated nucleic acid probe comprising an oligonucleotide whose sequence is at least 95% identical to a fragment, portion or segment of a polynucleotide encoding a polypeptide of the present invention. Such oligonucleotide probe may be either a DNA (i.e., a polydeoxyribonucleotide) or an RNA (i.e., a polyribonucleotide). In a preferred embodiment, said oligonucleotide probe and said fragment have the same sequence.

In a particular embodiment, said isolated nucleic acid probe will comprise an oligonucleotide that is at least 15 nucleotides in length, preferably at least 30 nucleotides in length, most preferably at least 60 nucleotides in length, and especially where said probe is at least 100 nucleotides in length. Such probes commonly hybridize to said oligonucleotides under stringent conditions, as defined herein. SEQ ID NO: 1. In another specific embodiment, the isolated nucleic acid probe oligonucleotide has the sequence of SEQ ID NO: 1.

In a separate embodiment, the isolated nucleic acid probe oligonucleotide of the present invention has a sequence at least 95% identical to the sequence, and is preferably identical to a sequence, selected from the group consisting of the sequences of SEQ ID NO: 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36.

The method of the present invention also provides a means wherein the polynucleotide coding for gpB protein is detected using a probe as disclosed herein. Useful probes also include oligonucleotides whose sequence is selected from the group consisting of SEQ ID NO: 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36.

Porcine gamma-herpesvirus specific oligonucleotides can be detected and/or prepared from the porcine gamma-herpesvirus gpB sequence of the present invention and can be synthesized according to known techniques. They will have substantial sequence identity (e.g., at least 75%, preferably at least 90%, most preferably at least 95%, and most especially 100% sequence identity) with one of the strands (either plus or minus) shown herein (SEQ ID NO: 23, which shows the sense, or plus, or coding, or anti-template strand) or an RNA equivalent, or with part of such a strand, or with a complement thereof.

The present invention further relates to isolated polynucleotides having at least 75% identity to the nucleotide sequence of SEQ ID NO: 23, preferably at least 90% sequence identity thereto, most preferably at least 95% sequence identity thereto, with the preferred embodiment being an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO: 23.

Likewise, polypeptides comprising the peptides encoded by porcine gamma-herpesvirus sequences are useful for generating antibodies to detect the presence of gamma-herpesvirus polypeptides when they are expressed in porcine tissues. Most useful is the polypeptide sequence of SEQ ID NO:24 (gpB protein) as well as immunogenically active fragments thereof (for example, the fragment whose sequence is that of SEQ ID NO: 2).

The present invention also relates to fragments, portions and segments of the polynucleotides and polypeptides disclosed herein, especially where said fragments, portions or segments are useful as probes (in the case of polynucleotides) or have immunogenic activity (in the case of polypeptides). Polypeptides of the present invention include fragments having at least 30, preferably at least 50, and most preferably at least 70 amino acid residues in common with some portion of the sequence of SEQ ID NO: 24.

"Polynucleotide sequences" as used herein refers to a chain of nucleotides such as deoxyribose nucleic acid (DNA) and transcription products thereof, such as RNA. The polynucleotides of the present invention include DNA, which includes cDNA, genomic DNA, non-genomic DNA, and synthetic DNA, and RNA, such as mRNA present in infected cells.

The term "oligonucleotide" encompasses nucleotides of preferably at least 15 bases (e.g. 15 bases to 600 bases) in length, more preferably 15 bases to 50 bases and most preferably 15 bases to 100 bases.

The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer sequences) as well as intervening sequences (introns) between individual coding segments (exons).

"Stringent hybridization" or "hybridization under stringent conditions" means hybridization that can be effected at a temperature of between 50°C and 70°C in 2X SSC (1X SSC is 17.5 g NaCl, 8.8 g of sodium citrate in 800 ml of $H_2O$, the pH is adjusted to 7.4 with NaOH and the volume adjusted to one liter), containing 0.1% sodium dodecyl sulfate (SDS). In a most preferred embodiment, the sample and probes are sufficiently similar that the hybridization is unaffected

by treatment with 0.1X SSC and 0.1% SDS at 65°C. Gamma-herpesvirus gpB specific oligonucleotides can be designed to specifically hybridize to gamma-herpesvirus specific nucleic acids. They can also be synthesized by known techniques and used as primers in PCR (i.e., polymerase chain reaction), or sequencing reactions, or as probes in hybridizations designed to detect the presence of gamma herpesvirus material in a sample. The oligonucleotides may be labeled by suitable labels known in the art, such as radioactive labels, chemiluminescent labels or fluorescent labels and the like.

In accordance with the present invention, the term "Percent Identity" or "Percent Identical", when referring to a sequence, means that a sequence is compared to a claimed or described sequence after alignment of the sequence to be compared (the "Compared Sequence") with the described or claimed sequence (the "Reference Sequence"). The percent identity is then determined according to the following formula:

$$\text{Percent Identity} = 100[1-(C/R)]$$

wherein C is the number of differences between the Reference Sequence and the Compared Sequence over the length of alignment between the Reference Sequence and the Compared Sequence wherein (i) each nucleotide or amino acid in the Reference Sequence that does not have a corresponding aligned nucleotide or amino acid in the Compared Sequence and (ii) each gap in the Reference Sequence and (iii) each aligned nucleotide or amino acid in the Reference Sequence that is different from an aligned nucleotide or amino acid in the Compared Sequence, constitutes a difference; and R is the number of nucleotides or amino acids in the Reference Sequence over the length of the alignment with the Compared Sequence with any gap created in the Reference Sequence also being counted as a nucleotide or amino acid. If an alignment

exists between the Compared Sequence and the Reference Sequence for which the Percent Identity as calculated above is about equal to or greater than a specified minimum Percent Identity then the Compared Sequence has the specified minimum Percent Identity to the Reference Sequence even though alignments may exist in which the hereinabove calculated Percent Identity is less than the specific Percent Identity.

Typically, the melting temperature ($T_m$) of an oligonucleotide less than 30 nucleotides may be calculated according to the formula:

$$T_m = 86.35 - 0.41\ [\%(G+C)] - 600/N$$

where N = Chain Length (i.e., number of base pairs)

The present invention also relates to vectors that include the novel polynucleotides (including fragments, segments and portions thereof, as defined below) disclosed herein, host cells which are genetically engineered with or without vectors of the invention to contain said polynucleotides and express said polypeptides, and the synthesis of polypeptides of the invention by recombinant techniques or by direct chemical synthesis.

As used herein, the terms "portion," "segment," and "fragment," when used in relation to polypeptides, refer to a continuous sequence of residues, such as amino acid residues, which sequence forms a subset of a larger sequence. For example, if a polypeptide were subjected to treatment with any of the common endopeptidases, such as trypsin or chymotrypsin, the oligopeptides resulting from such treatment would represent portions, segments or fragments of the starting

polypeptide. When used in relation to a polynucleotides, such terms refer to the products produced by treatment of said polynucleotides with any of the common endonucleases or exonucleases.

A polypeptide of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

The present invention further relates to a polypeptide which comprises the deduced amino acid sequence of SEQ ID NO: 24, as well as fragments thereof. Preferred are fragments comprising 25 or more consecutive amino acids, more preferred are fragments are fragments with at least 40 amino acids and even more preferred are fragments comprising 50 or more amino acids of the polypeptide of SEQ ID NO: 24. A preferred embodiment is the sequence of SEQ ID NO: 2.

The present invention further relates to variants of the disclosed polynucleotides which encode fragments, including analogs and derivatives, of the polypeptide comprising the amino acid sequence of SEQ ID NO: 24. Such variants may be naturally occurring allelic variants of the polynucleotides or may be non-naturally occurring (for example, variants produced by mutagenesis techniques).

Additional preferred embodiments include polynucleotides encoding gamma herpesvirus polypeptide variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, which comprise the amino acid sequence of SEQ ID NO:2 in which one or more of the amino acids

have optionally been replaced so long as said polypeptide still retains at least 80% identity with the amino acid sequence of SEQ ID NO 24, more preferably 90% sequence identity therewith, most preferably 95% sequence identity therewith and most especially being identical to the sequence of SEQ ID NO: 24, regardless of whether such sequence identities are achieved through addition, deletion, or substitution of amino acid residues.

Especially preferred among these are conservative substitutions, additions and deletions, which do not alter the properties and activities of the gamma herpesvirus gpB polypeptide. Also especially preferred are conservative substitutions. Most highly preferred are mature polypeptides comprising the amino acid sequence set forth in SEQ ID NO: 24 without substitutions.

Thus, the present invention includes polynucleotides encoding polypeptides comprising the sequence of SEQ ID NO: 24 as well as variants of such polynucleotides which variants encode a fragment, derivative or analog of the polypeptides set forth in SEQ ID NO: 24. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequences comprising the coding portion of the polynucleotide sequence shown in Figure 2 (of SEQ ID NO: 23). As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The present invention also encompasses polynucleotides which may be fused in the same reading frame to a polynucleotide sequence which aids in

expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory or signal sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence is a pre-protein and may have the leader sequence cleaved by the host cell to form the secreted form of the polypeptide.

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptides of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptides fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al. 1984. *Cell* 37:767).

The terms "derivative" and "analog" when referring to the polypeptides comprising the polypeptide as set forth in SEQ ID NO:24, means polypeptides which retain essentially the same biological function or activity as such polypeptides. Thus, an analog includes a pre-protein which can be secreted following cleavage of the pre-protein portion to produce an secretable polypeptide.

The polypeptides of the present invention may be recombinant polypeptides, natural polypeptides or synthetic polypeptides, preferably recombinant polypeptides.

The fragment, derivative or analog of the polypeptides comprising the amino acid sequence set forth in SEQ ID NO: 24 may be one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and

such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a pre-protein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The polypeptides of the present invention include polypeptides comprising the polypeptide of SEQ ID NO:2 or a fragment thereof, which fragment may be all or a portion of the polypeptide of SEQ ID NO:2, as well as polypeptides which have at least 80% similarity to such polypeptides, preferably at least 90% similarity, more preferably at least 95% similarity, and most preferably are

identical to polypeptides comprising the amino acid sequence of SEQ ID NO:2 and include portions or fragments of such polypeptides with such portion or fragment comprising at least 30 amino acids, preferably at least 40 amino acids and most preferably at least 50 amino acids. Preferred embodiments are fragments comprising 30 or more consecutive amino acids, more preferred are fragments with at least 40 amino acids and even more preferred are fragments comprising 50 or more amino acids of the polypeptide of SEQ ID NO:24, such as SEQ ID NO: 2 (which corresponds to residue numbers 484-678 of SEQ ID NO: 24 (shown in Figure 3).

As known in the art "similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. For such a determination, two amino acid sequences are compared along a stretch of their sequences, any gap (or gaps) introduced in one sequence to improve the alignment and similarity to the other sequences is counted as spaces of dissimilarity equal to the number of amino acids corresponding to the gap which are present in the second sequence, and the total number of similar amino acids are divided by the total number of amino acids present in the comparison area which counts the spaces of gaps as part of the comparison area.

Fragments or portions of the polypeptides of the present invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length polypeptides. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

The present invention also relates to vectors which include polynucleotides, and fragments thereof, of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of the invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing polypeptides by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the *E. coli. lac* or *trp*, the phage lambda PL promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila S2 and Spodoptera Sf9 animal cells such as CHO, COS or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention relates to recombinant constructs comprising one or more of the sequences as broadly described above. The

constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pBS, pD10, phagescript, psiX174, pBluescript - SK, pBSKS, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); pTRC99a, pKK2233, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host. Baculovirus systems are especially useful in practicing the present invention.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vdctors are pKK232-8 and pCM7. Particular named bacterial promoters include lacl, lacZ, T3, T7, gpt, lambda PRI PL and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene products encoded by the recombinant sequences. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), alpha-factor, acid phosphatase, or heat shock proteins, among others. The

heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by

appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS7 lines of monkey kidney fibroblasts, described by Gluzman (1981) *Cell*, **23**:175, and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa, 293 and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The polypeptides can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of

the expressed polypeptide. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The present invention also relates to diagnostic assays for detecting expression of the gamma-herpesvirus gpB polypeptide in various tissues. Assays used to detect levels of the gamma-herpesvirus gpB polypeptide in a sample derived from a host are well-known to those of skill in the art and include radioimmunoassays, competitive-binding assays, Western Blot analysis, ELISA assays and "sandwich" assay. An ELISA assay (Coligan, et al., Current Protocols in Immunology, 1(2), Chapter 6, (1991)) initially comprises preparing an antibody specific to the gamma-herpesvirus gpB polypeptide antigen, preferably a monoclonal antibody. In addition a reporter antibody is prepared against the monoclonal antibody. To the reporter antibody is attached a detectable reagent such as radioactivity, fluorescence or, in this example, a horseradish peroxidase enzyme. A sample is removed from a host and incubated on a solid support, e.g. a polystyrene dish that binds the proteins in the sample. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein like BSA (bovine serum albumin). Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to gamma-herpesvirus gpB polypeptide attached to the polystyrene dish. All unbound monoclonal antibody is washed out with buffer. The reporter antibody linked, for example, to horseradish peroxidase is then placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to the gamma-herpesvirus gpB polypeptide. Unattached reporter antibody is then washed out. Peroxidase substrates are then added to the dish and the amount of color developed in a given time period is a measurement of the amount of the gamma-herpesvirus gpB polypeptide, or fragments thereof, present in a given volume of sample when compared against a standard curve.

A competition assay may be employed wherein antibodies specific to the gamma-herpesvirus gpB polypeptide, or fragments thereof, are attached to a solid support, labeled gamma-herpesvirus gpB polypeptide and a sample derived from the host are passed over the solid support, and the amount of label detected. The label can be detected, for example, by liquid scintillation chromatography and can be correlated to a quantity of the gamma-herpesvirus gpB polypeptide present in the sample.

A "sandwich" assay is similar to an ELISA assay. In a "sandwich" assay the gamma-herpesvirus gpB polypeptide, or a suitable fragment thereof, is passed over a solid support and binds to antibody attached to a solid support. A second antibody is then bound to the gamma-herpesvirus gpB polypeptide. A third antibody which is labeled and specific to the second antibody is then passed over the solid support and binds to the second antibody and an amount can then be quantified.

The present invention also relates to compositions comprising immunogenic polypeptides, and active fragments thereof, disclosed according to the invention. Where intended for use in a clinical setting, such compositions will commonly contain the polypeptides, and active fragments thereof, suspended in a pharmacologically acceptable diluent or excipient.

The present invention further relates to the use of such compositions as vaccines, wherein said vaccines comprise immunogenically effective amounts of said compositions. Additionally, the invention contemplates a method of vaccinating a pig against a porcine, or swine, gamma-herpesvirus by administering to said pig the vaccine of the present invention.

The present invention also relates to a method of immunizing an animal, especially a pig, against a porcine gamma-herpesvirus, comprising administering to said pig an isolated polynucleotide encoding a polypeptide (or immunogenically active fragments thereof) according to the invention, such that the encoded polypeptide is eventually expressed in an immunogenically effective amount.

Pharmaceutical compositions, such as those designed to vaccinate, or otherwise induce active immunity, may be administered in a convenient manner such as by topical, intravenous, intraperitoneal, intramuscular, intratumor, subcutaneous, intranasal or intradermal routes. Such pharmaceutical compositions are administered in an amount which is effective for treating and/or prophylaxis of the specific indication.

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of a Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies binding the whole native

polypeptide. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

Antibodies specific to the polypeptide of the present invention may be employed as a diagnostic to determine the presence of a gamma-herpesvirus in tissue, which gamma herpesvirus expresses the gpB polypeptide (or a related polypeptide) in a sample derived from a host by techniques known in the art. Such antibodies may be useful to provide passive immunity in a host.

More specifically, the present invention relates to a method for creating, or otherwise producing or inducing, passive immunity in a pig comprising administering to said pig an immunogenically effective amount of one or more antibodies specific for the polypeptides, or fragments thereof, disclosed herein.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein (1975) *Nature*, **256**:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al. (1983) *Immunology Today* **4**:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al. (1985) in *Monoclonal Antibodies and Cancer Therapy,* Alan R. Liss, Inc., pp. 77-96), to mention only a few. Newer technologies present no obstacles to practicing the present invention.

Antibodies specific for the polypeptides disclosed herein may also be generated by genetically engineered cells transformed by the introduction into the genome of said cells, or by introduction of non-integrating vectors into said cells, of either polynucleotides alone, or vectors containing said polynucleotides, coding for said antibodies.

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention.

Such antibodies to the polypeptides of the present invention may be utilized to detect the presence or the absence of the polypeptides of the present invention. Thus, they are useful in an assay to verify the successful insertion of the polynucleotides of the present invention (as part of a construct) into a host cell. Thus, the protein encoded by the inserted polynucleotide according to the present invention, when expressed by the transformed host cell, serves as a "marker" for the successful insertion of the polynucleotide that can be detected by an antibody for the marker.

In general, antibodies against the polypeptides will be administered in an amount of at least about 10 mg/kg body weight and in most cases they will be administered in an amount not in excess of about 8 mg/kg body weight per day. In most cases, the dosage is from about 1 mg/kg to about 10 mg/kg body weight daily, taking into account the routes of administration, symptoms, etc.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various

restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid, or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 10 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

General procedures useful in practicing the methods disclosed herein can be found in, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), Wu et al, *Methods in Gene Biotechnology* (CRC Press, New York, NY, 1997), and *Recombinant Gene Expression Protocols*, in *Methods in Molecular Biology*, Vol. 62, (Tuan, ed., Humana Press, Totowa, NJ, 1997), the disclosures of which are hereby incorporated by reference.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (See Sambrook et al, supra).

In carrying out the procedures of the present invention it is of course to be understood that reference to particular buffers, media, reagents, cells, culture conditions and the like are not intended to be limiting, but are to be read so as to include all related materials that one of ordinary skill in the art would recognize as being of interest or value in the particular context in which that discussion is presented. For example, it is often possible to substitute one buffer

system or culture medium for another and still achieve similar, if not identical, results. Those of skill in the art will have sufficient knowledge of such systems and methodologies so as to be able, without undue experimentation, to make such substitutions as will optimally serve their purposes in using the methods and procedures disclosed herein.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight. In order to facilitate understanding of the invention the following examples providing certain frequently occurring methods and/or terms will be described.

## EXAMPLE 1

### Isolation and Sequence Analysis of Porcine Gamma-Herpesvirus Glycoprotein B Gene Sequences

**Primers:** Primers were synthesized for use in the amplification of pGHV-gpB gene sequences. Alignment of gpB protein sequences from several known gamma-herpesviruses (Figure 1) showed that there are four conserved regions (identified by underlining). Degenerate primers corresponding to these regions were synthesized (Table 1).

**Table 1**

R = A or G  Y = C or T  M = A or C  K = G or T  S = G or C  W = A or T
H = A or T or C  B = G or T or C
D = G or A or T  N = A or G or C or T  V = G or A or C  I = Inosine

F and R indicate whether the primers were in the sense or antisense direction respectively.

| **Degenerate Primers** | **Polypeptide Sequence** | **Sequence (5' to 3')** |
|---|---|---|
| RTT-F1 | Includes sequence RTTVNC | MGA ACA ACI GTY AAY TGY GA |
| RTT-F2 | Includes sequence RTTVNC | MGA ACA ACI GTY AAY TGY CT |
| RTT-F3 | Includes sequence RTTVNC | MGA ACA ACI GTY AAY TGY |
| QLIV-F4 | Includes sequence QXQF/YAY | CAR ITI CAR TWT GCM TAY GAC |
| QLIV-F5 | Includes sequence QXQF/YAY | CAR ITI CAR TWT GCM TAY G |
| NPTV-F6 | Includes sequence VMXS/T/AY | GTB ATG WSH AGV ATH TAY GG |
| NPTV-F7 | Includes sequence VMXS/T/AY | GTB ATG WSH GCV ATH TAY GG |
| NPTV-R1 | | SWC ATI ACR STI GTI GGR TT |
| FREYN-R3 | Includes sequence FREYN | TR IGY GTA RTA RTT RTA YTC YCT RAA |
| FREYN-R4 | Includes sequence FREYN | GTA RTA RTT RTA YTC YCT RAA |
| FREYN-R5 | Includes sequence FREYN | CTG RAA RTT RTA YTC YCG RAA |
| FREYN-R6 | Includes sequence FREYN | TG IGY CTG RAA RTT RTA YTC YCG RAA |

Primers were also designed to Epstein-Barr virus (EBV) for control and assay optimization purposes (Table 2). Primer names ending with an "F" are sense strand primers, primer names ending with an "R" are anti-sense strand primers.

**Table 2**

| **Epstein-Barr Virus Control Primers** | **Similar to:** | **Sequence (5' to 3')** |
|---|---|---|
| EBV-F2 | RTT-F2 | AGA ACT ACC GTC AAC TGC CT |
| EBV-F3 | RTT-F3 | AGA ACT ACC GTC AAC TGC |
| EBV-F4 | QLIV-F4 | CAG ATC CAA TTT GCC TAC GAC |
| EBV-F5 | QLIV-F5 | CAG ATC CAA TTT GCC TAC G |
| EBV-F6 | NPTV-F6 | GTC ATG TCC AGC ATC TAC GG |
| EBV-R1 | NPTV-R1 | GAC ATG ACG GTG GTT GGA TT |
| EBV-R3 | FREYN-R3 | TGC GCC TGG AAG TTG TAC TCC CGG AA |
| EBV-R5 | FREYN-R5 | CTG GAA GTT GTA CTC CCG GAA |

Oligonucleotides used to sequence the pGHV gpB gene were as follows:

| Sequencing Prim rs | Sequ nce (5' to 3') | Hybridizes To: |
|---|---|---|
| –47 Sequencing Primer | CGC CAG GGT TTT CCC AGT CAC GAC | TOPO-pCRII: bases 434 - 458 |
| M13 Reverse | CAG GAA ACA GCT ATG AC | TOPO-pCRII: bases 205 - 222 |
| TEF-14 | CAG GGA CGA GAA GAG GCT TA | pGHV gpB: bases 1989 - 2008 |
| TER-22 | ACA CCA GAG CAG CTC TAT G | pGHV gpB: bases 1513 - 1531 |
| TEF-23 | TAG CAC CAA TCA GTG AAG AAG AGC | pGHV gpB: bases 2399 - 2422 |
| TEF-24 | GCC AGT GAT ATG GTA CAC AGT G | pGHV gpB: bases 322 - 343 |
| TEF-25 | TAA CAG GTC ACT ATG GAA CAC ACG | pGHV gpB: bases 140 - 163 |
| TEF-26 | TTC TTT AAG ACT AAA CAC AGG TGG | pGHV gpB: bases 537 - 560 |
| TEF-27 | GGA GTG GTG AAG ATG ATC ATG | pGHV gpB: bases 815 - 835 |
| TER-28 | CCA TAA TGT TAG TGG ACA ATA TGA C | pGHV gpB: bases 993 - 1017 |
| TER-29 | ATG ACG CTG TGA TGT CAT TGG | pGHV gpB: bases 1073 - 1093 |
| TER-30 | GAT GCA CTG AGA AGC CTG AGA C | pGHV gpB: bases 1673 - 1694 |

**Isolation and Sequence Analysis of Porcine Gamma-Herpesvirus Glycoprotein B**

Equal amounts of genomic DNA from miniature swine #13432 and #13433 were pooled together. These animals (a/d haplotype) had been the recipients of bone marrow or stem cells from a/c haplotype animals and had been given cyclosporine treatment. The animals had both developed a lymphoma. Genomic DNA was extracted using Qiagen, Inc.'s QIAmp® Blood Kit (Chatsworth, CA). One hundred ng of the DNA pool was added to each polymerase chain reaction

(PCR) tube along with reagents. The final 50 μl reaction mixtures included 25 mM KCl, 10 mM Tris-HCl (pH 8.3), 3.5 mM $MgCl_2$ (Stratagene, Los Angeles, CA), 0.2 mM dNTP and 2.5 units of Amplitaq Gold® DNA polymerase (Perkin-Elmer Corporation, Philadelphia, PA). Several different combinations of forward and reverse primers were used (20 pmoles of each primer per reaction). These are summarized as follows:

| Forward Primer | Reverse Primer |
|---|---|
| QLIV-F4 | FREYN-R5 |
| EBV-F4 | FREYN-R5 |
| QLIV-F4 | EBV-R5 |
| QLIV-F4 | FREYN-R6 |
| QLIV-F5 | FREYN-R5 |
| EBV-F5 | FREYN-R5 |
| QLIV-F5 | EBV-R5 |
| QLIV-F5 | FREYN-R6 |
| EBV-F4 | FREYN-R3 |
| EBV-F4 | FREYN-R4 |
| QLIV-F4 | FREYN-R3 |
| QLIV-F4 | FREYN-R4 |
| QLIV-F5 | FREYN-R3 |
| QLIV-F5 | FREYN-R4 |

The reactions were amplified in a Perkin-Elmer GeneAmp® 9600 thermal cycler. The initial denaturing step was 9 minutes at 95°C (required to activate the "hot-start" Amplitaq Gold®) followed by 30 cycles of 94°C for 30 seconds, 45°C for 60 seconds and 72°C for 60 seconds. Thermal cycling was followed by a 5 minutes incubation at 72°C and brought down to 4°C.

The PCR products were visualized on a 2% agarose gel stained with ethidium bromide. PCR products were visible using the following primer pairs: QLIV-F5 / FREYN-R6, EBV-F4 / FREYN-R4, QLIV-F4 / FREYN-R4, QLIV-F5 / FREYN-R3. The sizes of the product varied from approximately 350 base pairs to 800 base pairs (expected size of the product was approximately 600 base pairs). The PCR products were purified using Microspin G-50® columns (Amersham Pharmacia Biotech, Newark, NJ) and TA-ligated into the pCRII-TOPO® vector (Invitrogen Corp., San Diego, CA). The ligation reactions were then transformed into competent TOP10F' *E.coli* supplied by Invitrogen Corp. The cells were incubated on carbenicillin (Sigma Chemical Company, St. Louis, MO) / IPTG / X-gal (Amresco, Inc., Solon OH) agar plates and selected colonies were grown up in LB broth (Gibco Life Technologies, Baltimore, MD). Plasmid DNA was extracted using the Wizard® miniprep kit (Promega Corp., Madison, WI). *EcoRI* (New England Biolabs, Beverly, MA) restriction digests of the minipreps were electrophoresed on a 2% agarose gel to determine the insert size.

In order to screen for herpesvirus sequences, miniprep DNA from the clones was hybridized to an EBV probe in a slot-blot array. Miniprep DNA (1 μl of each sample tested) was denatured by adding NaOH followed by a 10 minute incubation at 96°C. The samples were then added to GeneScreen® membrane (NEN Life Sciences, Pittsburgh, PA) inserted in a Minifold II® slot-blot apparatus (Schleicher & Schuell, Keene, NH). The blot was removed and crosslinked using a UV Stratalinker 1800® (Stratagene). EBV PCR product was generated from an EBV-transformed human B cell line (721.221, ATCC CRL 1855) using similar reagents and conditions as the previous PCR and with EBV-F4 and EBV-R5 primers. The PCR product was denatured and added to the Ready-to-go Beads® random priming kit (Amersham Pharmacia Biotech, Newark, NJ) with $^{32}$P dCTP (NEN Life Sciences). Approximately 1 x $10^6$ CPM of probe in 10 ml of ExpressHyb® hybridization solution (Clontech Laboratories, Inc., Palo Alto, CA)

was added to a tube containing the slot-blot membrane and incubated at 60°C for 90 minutes. The probe was then removed and the membrane was washed twice for 10 minutes with 6X SSC at 60°C. 8 x 10" Fuji RX film (Fisher Scientific, Pittsburgh, PA) was exposed to the blot overnight and developed. Several clones from the PCRs using EBV-F4 / FREYN-R4 primers and QLIV-F4 / FREYN-R4 hybridized to the EBV probe. Clones from other primer pairs as well as a QLIV-F4 / FREYN-R4 clone with a uniquely small insert did not hybridize to the probe. Three EBV-F4 / FREYN-R4 EBV-positive clones and three QLIV-F4 / FREYN-R4 EBV-positive clones were selected for DNA sequencing. The DNA sequencing analysis was performed by Lark Technologies, Inc (Houston, Tx). The DNA sequence obtained is shown in Figure 2. The hypothetical protein sequence for the fragment of pGHV-gpB is presented in Figure 3. The sequences were analyzed using the National Center for Biotechnology Information's BLAST database search program accessible via the internet at www.ncbi.nlm.nih.gov.BLAST (Altschul *et al.*, 1997). pGHV-gpB was most closely aligned to Alcelaphine (wildebeest) herpesvirus 1 L-DNA (Genbank Accession Number AF005370). Comparison of pGHV-gpB sequence to SHV1 and SHV2 sequences indicated only low sequence similarity at either the nucleic acid of protein levels (Figures 4-7). Figures 8 and 9 show a comparison of the nucleic acids sequences of SEQ ID NO:1 and a portion of the porcine gamma herpesvirus polymerase (AF118399 and AF118401). Figure 10 shows a Blast 2 sequence comparison of the nucleic acid sequence of pGHV-gpB and Acelaphine herpesvirus (AF005370). Figure 11 shows a comparison of the protein sequences of pGHV-gpB and Acelaphine herpesvirus (AF005370).